STABLE LIQUID CHROMATOGRAPHY METHOD FOR THE RESOLUTION AND QUANTIFICATION OF RELATED IMPURITIES OF FOSAMPRENAVIR IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Highly resolved and validated Liquid Chromatography method was developed for the separation and quantification of fosamprenavir and its related impurity 2 and 5 in bulk and pharmaceutical formulations. Separation of fosamprenavir and its impurities was achieved on Prontosil ODS C18 column using mobile phase composition of methanol and 0.1 M sodium acetate in the ratio of 40:60 (V/V) at pH 5.9 as mobile phase at a flow rate of 0.9 mL/min in isocratic condition. UV detection of the eluents was monitored at a wavelength of 246nm. In these conditions, well resolved peaks were observed at a retention time of 8.67, 5.73 and 4.00 min for fosamprenavir, Impurity 2 and 5, respectively. Calibration curve was plotted in the concentration range of 75-450 µg/mL for fosamprenavir and 1-6 µg/mL for impurity 2 and 5. Forced degradation study confirms that the method can separate the known and unknown impurities of fosamprenavir and the % degradation was found to be very less in all the stress conditions. Hence the method is suitable for the identification and quantification of impurities 2 and 5 along with fosamprenavir in bulk drug and formulations.

Keywords: Fosamprenavir, HPLC Stability Study, Related Impurity 2, Related Impurity 5

INTRODUCTION

Fosamprenavir ([(3*S*)-oxolan-3-yl] *N*-[(2*S*,3*R*)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl) amino]-1-phenyl-3-phosphonooxybutan-2-yl]carbamate) (Fig.1) belongs to a group of HIV drugs called protease inhibitors (PIs), which is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection[1]. Protease inhibitors block a HIV enzyme called protease which prevents HIV from multiplying and can reduce the amount of HIV in the body[2].

The drug is available as tablets and oral suspension. Tablets are available for oral administration in the strength of 700 mg of fosamprenavir as fosamprenavir calcium. Oral suspension is available in the strength of 50 mg per mL of fosamprenavir. Serious side effects of fosamprenavir include severe skin reactions or rash. Other possible side effects include liver problems, diabetes and high blood sugar (hyperglycemia), changes in immune system, increase in body fat, changes in blood test results, increased bleeding in some people with hemophilia, kidney stones[3].

The present work is aimed at development and validation of an analytical method for fosamprenavir and with its impurities 2 and 5 (Fig. 2). The molecular formula of fosamprenavir is $C_{25}H_{36}N_3O_9PS$ where as $C_{19}H_{32}N_2O_3$ ((1R,2R)-1-Benzyl-2-hydroxy-3-isobutylamino-propyl)-carbamic acid tert-butyl ester Molecular Weight : 336.469) and $C_{25}H_{34}CaN_3O_9PS$ ([2R,3S)-1-[(4-aminophenyl) sulfonyl-(2-methylpropyl)amino]-3-[[(3S)-oxolan-3yl] oxycarbonylamino]-4-phenyl-butan-2-yl]oxyphosphonic acid Molecular Weight:623.669) are impurity 2 and 5 respectively. The literature review reveals that only few RP-HPLC methods were reported with fosamprenavir[4-7]. Hence the present work is an attempt to develope a novel method for the resolution and quantification of Fosamprenavir and its impurities.

MATERIALS AND METHODS

Instrumentation

The separation and quantification of related impurities of fosamprenavir was carried on Prontosil ODS C18

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column (250 mm x 4.6 mm, 5µ) equipped in isocratic PEAK HPLC instrument with LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector for detection. The samples were injected using Rheodyne manual inject port and data was analyzed by using PEAK software version 2.0. The standard and samples were weighed in DENVER (SI-234) electronic balance and ultrasonic batch sonicator (1.5 L) was used for the mixing and preparation of mobile phase, standard and samples.

Chemicals and Reagents

Fosamprenavir active pharmaceutical ingredient (API) and its two impurities 2 and 5 were obtained from GlaxoSmithKline India limited, Mumbai. The marketed formulation LEXIVA -700mg was purchased at local pharmacy. Laboratory reagent grade sodium acetate, acetic acid and HPLC grade methanol and water were purchased from Merck chemicals, Mumbai. The membrane filter papers (0.2µ nylon) were purchased from Millipore (India).

Preparation of Fosamprenavir Standard, Impurity 2 and 5 Solutions

25 mg of standard drug fosamprenavir was weighed accurately and dissolved in 25 mL methanol to obtain a solution at a concentration of 1000 µg/mL and the solution was filtered. A standard solution of fosamprenavir at a concentration of 100 µg/mL was prepared by diluting 10 mL from 1000 µg/mL to 100 mL. The same procedure was used for the preparation of standard solutions of impurity 2 and 3 separately. Equal volumes of fosamprenavir, Impurity 2 and 5 , of known concentrations were mixed to get mixed standard solution. This combined solution of fosamprenavir, Impurity 2 and 5 of known concentrations was used for method development and validation study.

Preparation of Formulation Solution:

The market formulation tablets of fosamprenavir with brand LEXIVA© -700mg was powdered using sterile mortar and pestle. An amount of the tablet powder equivalent to 10mg of fosamprenavir was weighed accurately and was dissolved in 10 mL methanol. Then it was filtered and 3 mL of this solution was again diluted to 10 mL to get a formulation solution having 300 µg/mL of fosamprenavir. This solution was used for the estimation of fosamprenavir and its related impurities in pharmaceutical formulations using HPLC developed method.

Method Development

Different method development trails were performed to arrive at a suitable method development for the identification and quantification of fosamprenavir and its related impurities 2 and 5 in pharmaceutical formulations. In this process, composition of mobile phase, pH of mobile phase, configuration of stationary phase, UV detector wavelength and mobile phase flow rate was studied. In each trail condition, the system suitability parameters were checked and the conditions that produce best results were considered as optimized.

METHOD VALIDATION

The method was validated in terms of determination of range of analysis, sensitivity, accuracy, precision, ruggedness, robust nature of the method developed for the separation and quantification of Fosamprenavir along with its Impurities 2 and 5.

Forced Degradation Studies:

Forced degradation study was carried for the standard drug fosamprenavir in the developed method to evaluate the effectiveness of the developed method for the separation and identification of known and unknown impurities in the drug[8-9]. 50mg of standard drug fosamprenavir was mixed with 50mL of 0.1N HCl for acid hydrolysis study, 50mL of 0.1N NaOH in base hydrolysis study and 50mL of 3% hydrogen peroxide solution for oxidative degradation study. The solution was incubated for 24h and then neutralized. The neutralized standard drug was diluted to300 µg/mL and was analyzed in the developed method condition. In photolytic and thermal degradation conditions, standard drug was kept under UV light at 254nm and in oven at 60^{0}C for 24h, respectively. Then the standard drug was diluted to 300µg/mL and was analyzed in the developed method condition. The % degradation, number of degradation products formed

Table I : System Suitability Results

Parameter	Fosamprenavir	Impurity 2	Impurity 5
API Concentration	300 µg/mL	4µg/mL	4µg/mL
RT (min)	8. 67	5.73	4.00
Area	904139.8	50193.7	58746.9
Resolution	11.23	4.18	---
Theoretical Plates	7415	5124	3746
Tailing Factor	1.26	0.91	1.15

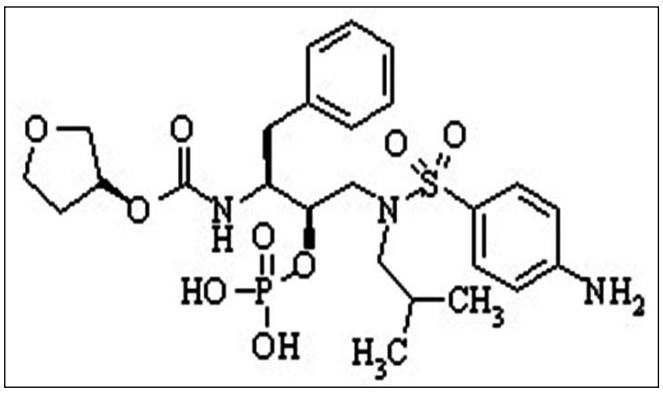

Fig. 1: Fosamprenavir Molecular Structure

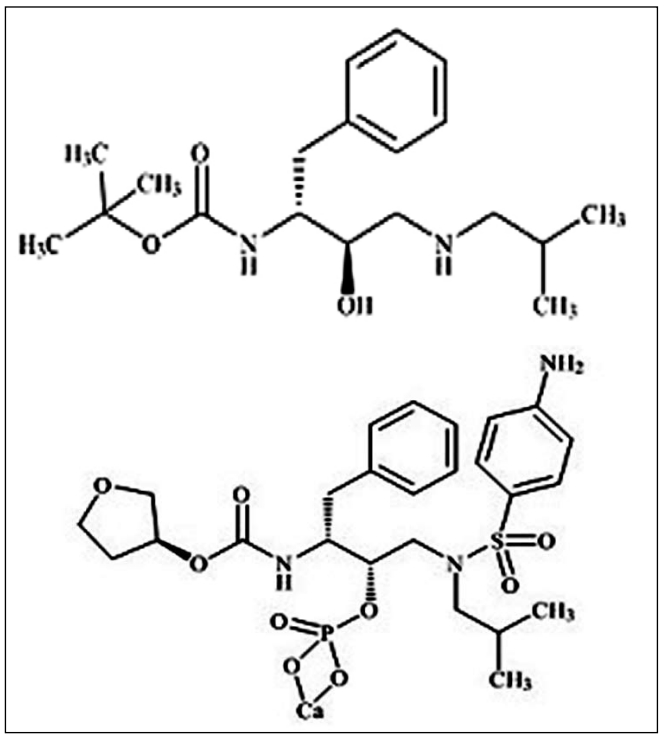

Fig. 2: Fosamprenavir Related Impurity 2 and 5 in the study

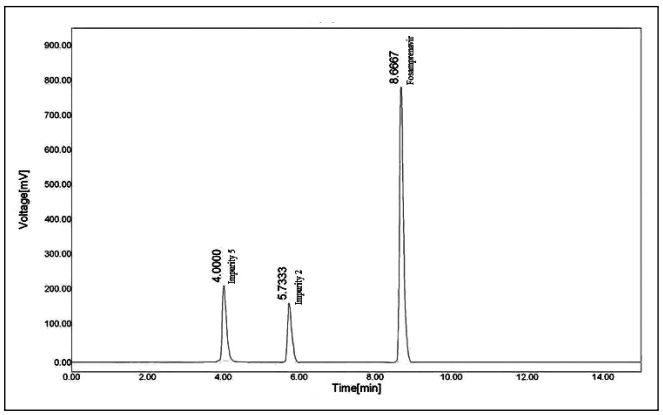

Fig. 3: Standard chromatogram in the optimized conditions

in the degradation study and the % effectiveness of the method for the separation of degradation products were evaluated.

Formulation Analysis

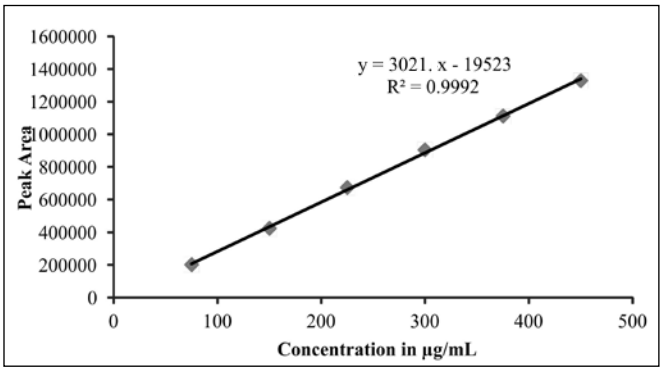

Fig. 4: Linear calibration curve of Fosamprenavir

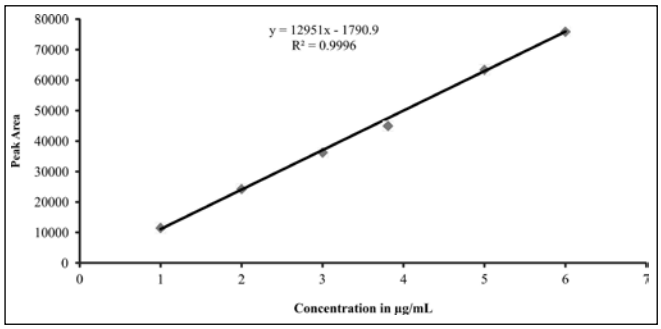

Fig. 5: Linear calibration curve of impurity 2

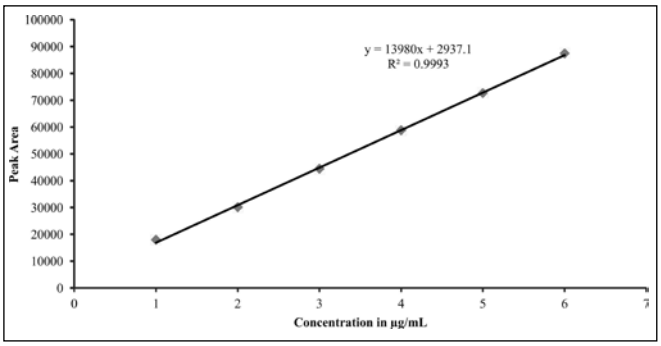

Fig. 6 : Linear calibration curve of impurity 5

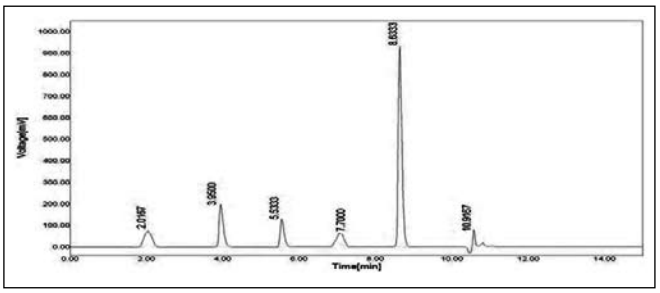

Fig. 7: Acid hydrolysis chromatogram

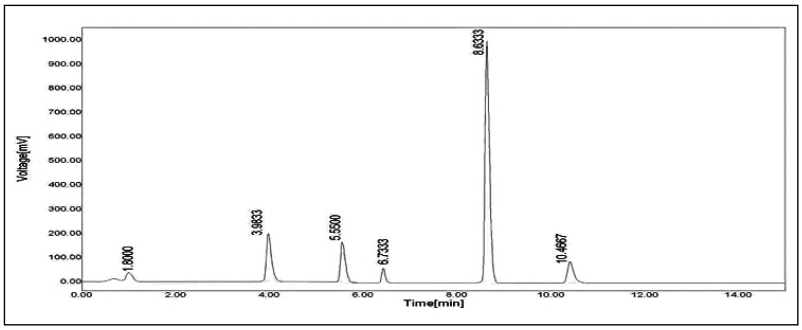

Fig . 8: Base hydrolysis chromatogram

The formulation solution prepared from the formulation tablets of fosamprenavir (LEXIVA© -700mg) was analyzed in the developed method. The % assay of the fosamprenavir in the developed method was calculated.

RESULTS AND DISCUSSION

As the literature survey indicates that there is no HPLC method reported for the analysis of

Table II: Linearity Results

S. No	Fosamprenavir		Impurity 2		Impurity 5	
	Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area
1	75	200586	1	11458	1	17928
2	150	424429	2	24203	2	30104
3	225	672884	3	36187	3	44398
4	300	904139	4	50193	4	58746
5	375	1110706	5	63350	5	72599
6	450	1328793	6	75823	6	87420

Table III: Accuracy Results

S. No	Compound	Level	Concentration in µg/ml				% Recovery	% RSD
			Target	Spiked	Total	Recovered Mean±SD		
1	Fosamprenavir	50%	150	75	225	224.44±0.34	99.75	0.15
2		100%	150	150	300	297.15±0.64	99.05	0.21
3		150%	150	225	375	369.79±1.21	98.06	0.32
4	Impurity 2	50%	2	1	3	2.98±0.004	99.59	0.13
5		100%	2	2	4	3.96±0.008	99.24	0.20
6		150%	2	3	5	4.98±0.008	99.64	0.16
7	Impurity 5	50%	2	1	3	2.95±0.007	98.53	0.23
8		100%	2	2	4	3.97±0.011	99.38	0.29
9		150%	2	3	5	4.94±0.007	98.83	0.14

Table IV: Forced Degradation Results

S. No	Condition	No of additional peaks	Peak Area	% recovered	% degraded
1	Acid hydrolysis	3 + 2 Impurities studied	810353	89.62	10.37
2	Base hydrolysis	3 + 2 Impurities studied	804529	88.98	11.01
3	Oxidative degradation	3 + 2 Impurities studied	844716	93.42	6.57
4	Thermal	2 + 2 Impurities studied	864923	95.66	4.33
5	Photolytic	1+2 Impurities studied	820394	90.74	9.26

fosamprenavir and its related impurities in bulk drug and pharmaceutical formulations, the present work is aimed to develop a simple and precise HPLC method for the estimation of fosamprenavir and its related impurities 2 and 5. All the method development and validation studies were carried as per the ICH guide lines[10-11].

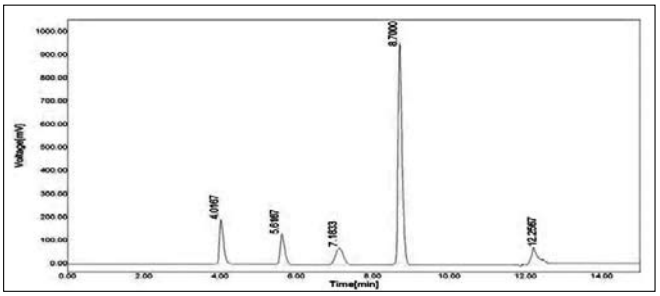

Fig. 9 : Oxidative degradation chromatogram

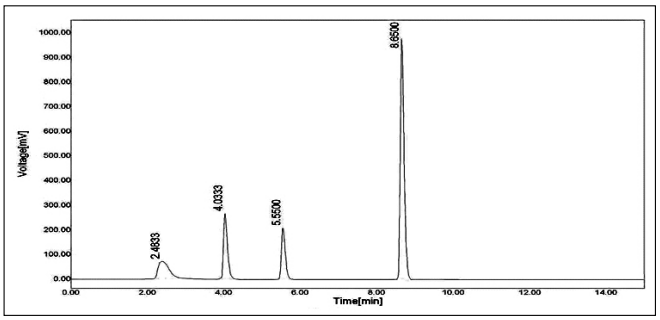

Fig. 10: Thermal degradation chromatogram

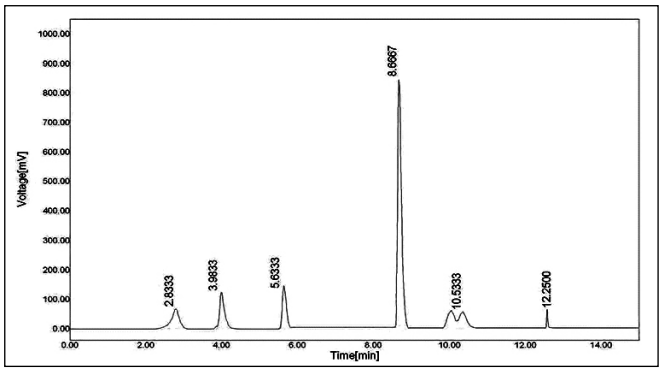

Fig. 11: Photolytic degradation chromatogram

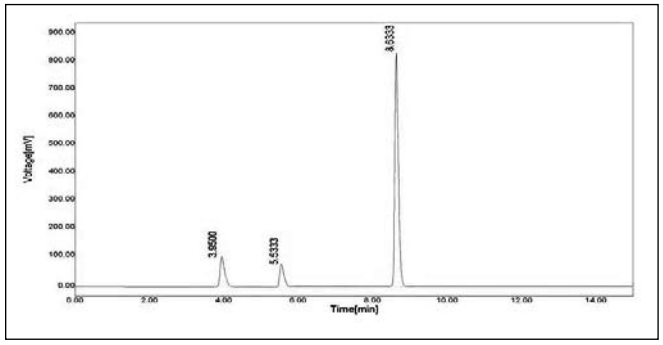

Fig. 12: Formulation chromatogram

The optimized separation was achieved using isocratic 0.9mL/min flow rate of mobile phase methanol and 0.1M sodium acetate in the ratio of 40:60 (V/V) at pH 5.9 with 1% acetic acid. Prontosil ODS C18 column is used as stationary phase and UV detection was monitored at 246nm.

In the optimized conditions, the standard drug and impurities were well resolved and retained at a retention time of 8. 67 min, 5.73 min and 4.00 min for fosamprenavir, Impurity 2 and Impurity 5 respectively and a clear base line was observed. The method obeys system suitability conditions (Table I) for both standard and impurities. Fig. 3 shows the optimized chromatogram of fosamprenavir and its related impurities 2 and 5 in the developed method.

Accurately correlated calibration range was observed in the concentration range of 75-450 µg/mL for fosamprenavir, 1-6 µg/mL for impurity 2 and 5 respectively.

The regression equation was found to be y = 3021.x – 19523 (R^2 = 0.999), y = 12951.x – 1790 (R^2 = 0.999) and y = 13980x + 2937 (R^2 = 0.999) for fosamprenavir, impurity 2 and 5 respectively.

The calibration curve was found to be linear in the concentration studies for both fosamprenavir and its related impurities considered.

The results of linearity study were given in Table II and calibration curves were shown in Fig. 4 to 6 for fosamprenavir, impurity 2 and 5, respectively.

The repeatability and reproducibility was studied by intraday, interday precision and ruggedness study. The standard solution at a concentration of 300 µg/mL of fosamprenavir, 0.6 µg/mL impurities 2 and 5 each were analyzed six times in the same day for intraday precision, six times in three successive days for interday precision and six times for change in analyst for ruggedness study. The % RSD in each changed condition was calculated and found to be 0.151, 0.402 and 0.564 in intraday precision, 0.131, 0.903 and 0.202 in interday precision and 0.261, 0.662 and 0.428 in ruggedness study for fosamprenavir, impurity 2 and 5, respectively. This confirms that the method developed was found to be precise and rugged for the analysis of fosamprenavir, impurity 2 and 5.

The standard concentration of fosamprenavir, impurity 2 and 5 were analyzed by change in analytical conditions.

The % change was calculated and was found to be within the acceptable limit of less than 1 for fosamprenavir, impurity 2 and 5 which confirms that the method was found to be robust. The spiked recovery at 50%, 100% and 150% spiked levels at a target concentration of 150 µg/mL of fosamprenavir and 2 µg/mL of impurity 2 and 3 were studied. The % Recovery and the % RSD of recovery in each spiked level for fosamprenavir, impurity 2 and 5 were calculated (Table III) and was found to be within the acceptable limits and confirms that the method to be accurate.

The sensitivity of the method was confirmed by detection and quantification results of fosamprenavir, impurity2 and 5. The LOD of the method was determined using standard deviation of the response (s) and the slope of the calibration curve (m) values in using the formula (LOD=3.3s/m). The LOQ was calculated from the obtained LOD values (LOQ=3.3×LOD). Very sensitive detection limits of 4.3µg/mL, 0.16µg/mL and 0.23µg/mL and quantification limits of 1.3µg/mL, 0.05µg/mL and 0.07µg/mL were obtained for fosamprenavir, impurity 2 and 5 respectively.

In acid hydrolysis condition, the % degradation of 10.37 % was observed with three additional degradation compounds (Fig. 7). A very high % degradation of 11.01 with three additional degradation compounds was observed in base hydrolysis degradation study (Fig. 8). 6.57%, 4.33% and 9.26% of degradation was observed in oxidative degradation (Fig.9), thermal (Fig. 10) and photolytic degradations (Fig.11) respectively. In all these degradation conditions, impurity 2 and 5 were detected along with fosamprenavir and the additional degradation compounds formed during the stress study were also effectively separated which confirms that the method was found to effectively separate known and unknown impurities in fosamprenavir. Table IV shows the forced degradation study results of fosamprenavir in different stress conditions.

The formulation assay was found to be 99.20% for fosamprenavir in the developed method. In the formulation chromatogram, both the impurities were detected and clear base line was observed. That no excepients detected in the formulation chromatogram confirms that the method was suitable for the routine analysis of fosamprenavir. The formulation chromatogram was shown in Fig. 12.

CONCLUSION

A simple and stable RP HPLC method was successfully developed and validated for the separation, qualitative and quantitative analysis of fosamprenavir and its related impurities 2 and 5. All the validation parameters were found to be within the limits and can effectively separate the known and unknown impurities forced during the stress study. Hence the method can be used for the routine analysis of fosamprenavir and its related impurities 2 and 5 in furnished products and in formulations.

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